Aeromonas salmonicida associated with Hetrophyes hetrophyes infection in a Sacred Ibis (Threskiornis aethiopica) flock: A Case Report

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Abstract: *Aeromonas (A. salmonicida*) belonging to the genus *Aeromonas*, is a common pathogen that causes furunculosis and septicaemia in variety of fishes. It infects cold blooded vertebrates living at low temperatures mainly salmonid fish hence named salmonicida. Untill recently *A. salmonicida* is considered to be a fish pathogen. Sudden deaths occurred among 5 out of 31 birds of a flock of Sacred Ibis (Threskiornis aethiopica) at the zoo of Giza, Egypt in February 2016 after ingestion of a tilabia zilli fish meal. The birds aged over 10 years old and deaths occur within few hours of ingestion. Post-mortem examination identified dark blackish localized colorization on the outer surface of the lung and turbid serous membranes. Dark red coloration of liver with focal areas of attached turbid capsule besides, the gizzard and intestine contain dark red content and brain was tangled with red coloration. Parasitological examinations confirmed the presence of severe infection with Hetrophyes hetrophyes. Bacteriological examination revealed the isolation of A. salmonicida from the brain of dead bird. This is the first demonstration of A. salmonicida associated with mortality in a wild bird in Egypt. The significance of A. salmonicida infection in wild birds, and its implications for poultry and captive bird health, is currently unknown.
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Keywords: Sacred Ibis, Aeromonas salmonicida, Hetrophyes hetrophyes

Introduction

The African Sacred Ibis (Threskiornis aethiopicus) is a species of wading bird of the ibis family, Threskiornithidae, which breeds in sub-Saharan Africa, southeastern Iraq, and formerly in Egypt, where it was venerated and often mummified as a symbol of the god Thoth. Sacred ibis (Threskiornis aethiopicus) is a large, white plumage short-legged ibis having a black, naked head and neck. The sacred ibis is specialized by nature for aquatic prey like small fish and invertebrates, but is an opportunistic eater that will take anything available, such as carrion, bird eggs and nestlings, or small mammals. The Sacred Ibis is one of the species to which the Agreement on the Conservation of African-Eurasian Migratory Water birds (AEWA) applies.

Heterophyiosis is infection with the intestinal fluke Heterophyes heterophyes, which is acquired by eating infected raw or undercooked fish from fresh water or brackish water. Heterophyes heterophyes is endemic in the Far East, Middle East, and Egypt. The life cycle of heterophyid parasites includes snails as a first intermediate host, several fish species as the second intermediate host, containing the larval encysted metacercariae (EMC), and fish-eating birds and mammals including humans as a definitive host, harboring the adult fluke causing heterophyiosis **(Elsheikha and Elshazly 2008; Simões et al., 2010).**

Infection is acquired by eating metacercariae (encysted stage). After ingestion, metacercariae excyst and attach to the mucosa of the small intestine. There, they develop into adults; growing to about 1.0 to 1.7 mm by 0.3 to 0.4 mm. Infection with Metagonimus yokogawai, a related trematode, has been reported after eating raw or undercooked freshwater or brackish fish

Aeromonas salmonicida is belonging to the genus Aeromonas and is a common pathogen that causes furunculosis and septicaemia in variety of fishes. It infects cold blooded vertebrates living at low temperatures mainly salmonid fish hence named salmonicida (**Wiklund and Dalsgaard 1998**). Untill recently Aeromanas salmonicida is considered to be a fish pathogen. Previously other Aeromonas species like A. hydrophila, A.caviae, A. veronii etc were also considered as pathogen in cold blooded animals only, including fish, amphibians and reptiles but gradually recognised as opportunistic pathogen for humans. However these organisms have increasingly been identified as a primary pathogen for humans in normal individual as well as in immunocompromised patient mainly in gastrointestinal infections and septicaemia (**kao et al., 2003**).

2. Material and Methods

Case presentation:

Sudden deaths among 5 out of 31 birds of Sacred Ibis (Threskiornis aethiopica) flock in the zoo in Giza, Egypt in February 2016 were occurred after ingestion of a tilabia zilli fish meal. The birds aged over 10 years old and deaths occur within few hours of ingestion. There was a history of sporadic cases of diarrhoea among the flock suggestive of immunocompromised. Dead birds were brought to poultry diseases department- animal health research institute – Giza Egypt for diagnosis the cause of death.

Pathological Examination:

Pathological examination of the recovered carcass (the first case seen) was performed using a standardized protocol comprising systematic external and internal examination of body systems. Collected Organs were from liver, brain and lung. The specimens for histopathological examination were fixed in 10% neutral formalin and processed by routine histology techniques (**Dzhurov et al., 1989 and Dyakov et al., 1989**). Cross sections (4 µm) were stained with haematoxylin-eosin (H&E).

Parasitological Examinations

For parasitological examinations, the feathered and unfeathered parts of the bird were thoroughly examined for any external parasites. For detection of internal parasites, the trachea, esophagus, crop, proventriculus, gizzard and intestine are thoroughly examined for the presence of helminthes macroscopically and microscopically. Specimens were identified according to **Yamaguti (1971**). Smears from the intestinal contents and mucosa were examined for enteric and any minute helminths, eggs, or larvae either directly with one drop of normal saline or by the concentration floatation technique (**Levine, 1985**). Thin mucosal scrapings were obtained from conjunctiva, nasal sinuses trachea, and different parts of the intestine and fixed with methanol for 10 minutes, stained with the Modified Ziehl-Neelsen staining technique (**Henriksen and Pohlenz, 1981**) and examined by oil immersion lens for cryptosporidium infection.

Bacteriological Examination:

For bacteriological examination samples were taken from the brain to avoid contamination with contaminant and saprophytic bacteria. Swabs prepared from these organs were inoculated directly onto brain heart infusion agar and MacConkey agar plates. The cultures were then incubated aerobically **at 37°C for 24-48 hours. Brain swab was also** cultured into brain heart infusion broth (BHI) then incubation of the broth mixture at 37°C for 24 h followed by sub-cultured onto blood, XLD and MacConkey agar plates. Biochemical identification was carried out according to **Holt et al (1994), USFWS/ AFS-FHS (2004) and USFWS/ AFS Fish Health Centers (2008).** Antibiotic **sensitivity** was determined by Kirby Bauer's disk diffusion method as performed in **CLSI guidelines (2009).**

Results

Post-mortem examination of the recovered carcass identified dark blackish localized colorization on the outer surface of the lung, turbid serous membranes. Dark red coloration of liver with focal areas of attached turbid capsule, the Gizzard and intestine contain dark red content. The brain was tangled with red coloration. The histopathological examination of Brain showed Cerebral congested blood vesseles (fig.1) pronounced perivascular edema, Gliosis (fig 2) and Spongiosis with different degree of severity (fig. 3).

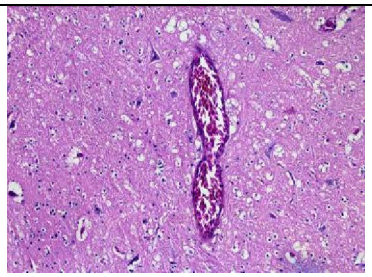

Fig. 1 Brain showed congested blood vesseles H&E X 250.jpg

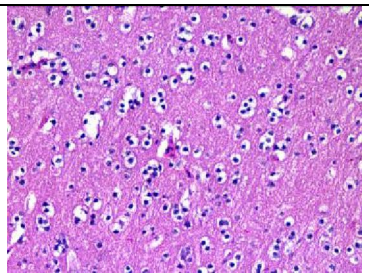

Fig. 2 - Brain showed glosis H&E X 250.jpg

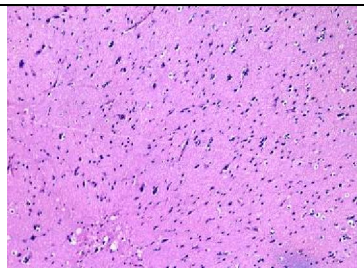

Fig. 3. Brain showed spongiosis H&E X 400.jpg

Lung showed severe congestion of pulmonary blood vessels, Emphysema in addition of anthracois (fig. 4 & fig. 6) and Perivascular and alveolar edema and also thrombosis (fig.5).

Liver revealed severe subcapscular congested blood vesseles, Focal haemorrahgic areas accompanied with fibrinous perihepatitis (fig.7), Degenerative changes of perivascular hepatocytes mainly with vacuolar changes (fig. 8) and distributed coagulative necrosis periportal and parenchymal inflammatory cells aggregations (fig 9), pronounced subcapscular inflammatory cells proliferation as well as invaded hepatocytes with cellular demarcation.

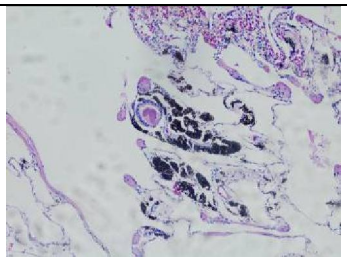

Fig. 4. Lung showed emphysema H&E X 250.jpg

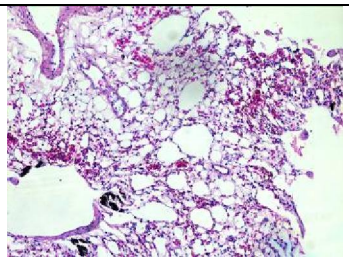

Fig. 5. Lung showed thrombus H&E X 100.jpg

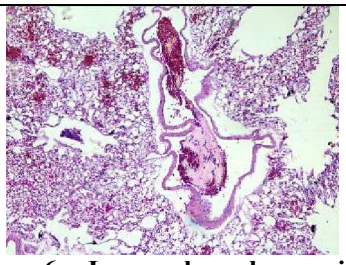

Fig. 6. Lung showed massive anthracosis H&E X100.

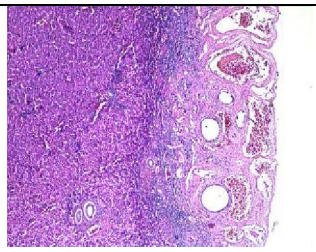

Fig. 7. Liver showed fibrinous inflammation H&E X 100.jpg

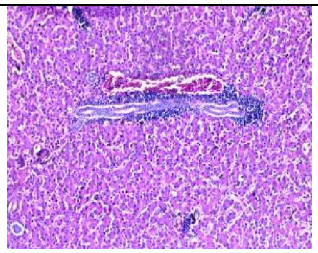

Fig. 8. Liver showed vacuolar degeneration H&E X 250.jpg

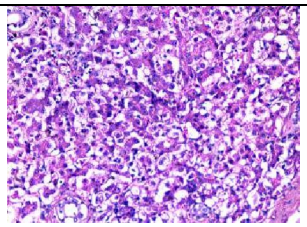

Fig. 9. Liver showed periportal inflammatory cells aggregation H&E X100.jpg

The parasitological examination revealed sever *Heterophys heterophys* infection which could be easily detected in the fresh smears and Heterophys eggs found mainly in groups. The fluke is minute teardrop or pear -shaped found in the small intestines. The adult flukes range from 1.1 mm to 2mm long and about 0.35 mm at their greatest width. The body of the fluke is covered with scales, mostly concentrated at the anterior end. Also at the anterior end, there is an oral sucker located in the medio-anterior of the body which sometimes armed by spines (Figure 10) or not armed (Fig. 11). At the posterior end of the fluke there are two oval testes. The fluke also has female reproductive organs, located medio-posterior winch of the ovary and leading away from the ovary, the vitellaria. The uterus is a long tube- like structure that also leads away from the ovary. Uterus may be filled with large number of eggs (Fig. 12). The eggs are small, operculatted, yellowish brown with thin shell, (Fig.13).

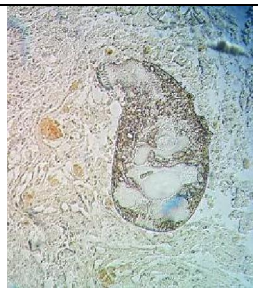

Fig. (10): The fluke showing the oral and ventral suckers with spines around the oral sucker the two oval testis and the overy.

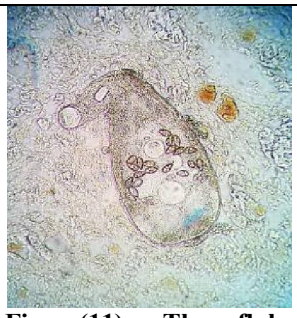

Fig. (11): The fluke showing the oral and ventral suckers without spines around the oral sucker

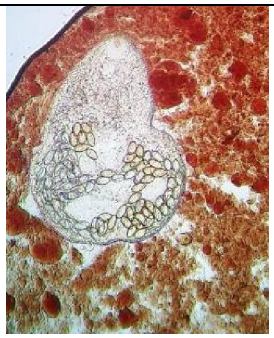

Fig. (12): Showing the uterus with large number of eggs.

Fig. (13): Eggs of Heterophys heterophys, yellow brown in colour and operculated

Bacteriological examination of the brain revealed the isolation of, round, shiny, brownish colored colonies produce melanin-like pigments on BHI agar (Fig.14), raised with β haemolysis on Blood agar (Fig.15), pale non-lactose fermenting brownish colonies on MacConkey agar, pink colonies on XLD agar (Fig. 16). The bacterial colonies were carefully selected and subjected to Gram staining prior to identification by cellular morphology.

Fig 14: *Aeromonas salmonicida* colonies on BHI agar

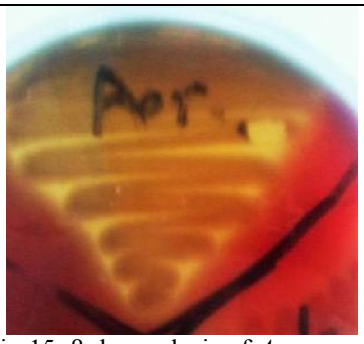

Fig 15: β -haemolysis of *Aeromonas salmonicida* on Blood agar

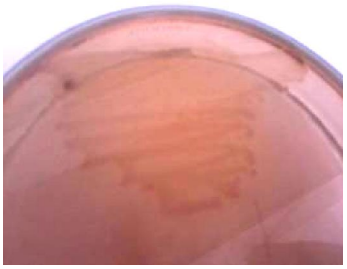

Fig 16: *Aeromonas salmonicida* pale non-lactose fermenting brownish colonies on MacConkey agar

Isolated organisms were presumptive confirmed as *Aeromonas salmonicida* biochemically according to **Holt et al (1994)**, **USFWS/ AFS-FHS (2004) and USFWS/ AFS Fish Health Centers (2008).** The isolated microorganism was non- motile and positive for oxidase, catalase, and gelatin hydrolysis, triple sugar iron (TSI) was K/K with production of hydrogen sulphide while negative for indole, urease, mannitol, esculine and simmons citrate. The isolated microorganism was found to be sensitive for colistin sulphate (25 mcg), doxycycline (30 mcg), cefotaxime (30 mcg), norfloxacin (5 mcg), enrofloxacin (5 mcg), gentamycin (10 mcg), nitrofurantoin (300 mcg), sulfamethxazole/ trimethoprim (25 mcg) while was resistant to amoxicillin (10 mcg), cefradine (30 mcg) and erythromycin (15 mcg) (Oxoid).

4. Discussion

Bacteria of the genus *Aeromonas* and Family *Aeromonadaceae* are Gram negative rods, asporogenous and non-lactose fermenting organisms. Up to fourteen *Aeromonas* species have been discovered compared with other pathogenic aeromonads. *Aeromonas salmonicida*, a non-motile aeromonad, is the aetiological agent of a bacterial septicaemia in fish, called furunculosis **(Bernoth, 1997 and Wiklund and Dalsgaard 1998).** *A. salmonicida* can also be found in environment, diseased fish and water and may be transmitted by all these sources.

In the present work *Aeromonas salmonicida* strain was recovered from the brain of case of sudden death among Sacred Ibis (Threskiornis aethiopica) flock. The microorganism was identified biochemically as *Aeromonas salmonicida.* The same biochemical results were recorded by **Kim et al. (2011)** to identify *Aeromonas salmonicida.* They considered the microorganism as primary pathogen in variety of fishes and mentioned that it cannot grow at 37ºC. **Mcintosh and Austin (1991)** reported that incubation of *Aeromonas salmonicida* at supra-optimal temperatures, i.e. 30-37 *"C,* resulted in the expression of motility by polar flagella, and changes in sugar fermentation patterns, e.g. loss of acid production from mannitol, loss of the ability to degrade complex molecules (aesculin, DNA, elastin and gelatin). On the other hand, **Tewari et al. (2014)** cultured the isolated of *Aeromonas salmonicida* from human blood sample by two different methods (conventional and BacT alert) and sub-cultured plates were incubated at 37ºC and for both samples (first and second) same colonies were isolated which suggests *A. salmonicida* can grow at 37ºC. **Abbott et al. (2003)** evaluated 193 strains representing 14 different Aeromonas genomospecies for 63 phenotypic properties and found that only 9 of 62 biochemical tests (14%) yielded uniform results, and the fermentation of certain carbohydrates was found to be linked to specific species.

In this study, the isolation of the microorganism from the brain of the bird following sudden death indicates the pathogenic ability of the microorganism and its role in inducing death among the infected flock. The microorganism was capable to induce β haemolysis on Blood agar culture medium. The virulence of Aeromonas is complex and involves multiple virulence factors such as various hydrolytic enzymes, cytotoxic and cytotonic enterotoxins and haemolytic toxins. **(Janda and Abbott, 2010**). The primary toxins produced are haemolysins, which is a heat-labile b-haemolysin, which exhibits phospholipase A and C activity. It is a pore-forming cytolysin able to diffuse into the bilayer cell membrane causing leakage of cytoplasmic contents. Haemolytic enterotoxins have been reported by some authors (**Chopra, et al., 1991; Gosling, 1996. Howard et al., 1996**) characterized by weak haemolysin, glycerophospholipid: cholesterol acyltransferase (GCAT) from *A. hydrophila* and *A. salmonicida.* In addition, at least one cytotonic enterotoxin with similar activity to cholera toxin has been demonstrated (**Ljungh et al., 1982; Gosling et al., 1993; Gosling, 1996**). Species of *Aeromonas*

produce a range of cell-surface and secreted proteases which probably enhance virulence (**Gosling, 1996**). These virulence factors enable the bacteria to colonize, gain entry, establish, replicate, and cause damage in host tissues and to evade the host defense system and spread, eventually killing the host (**Yu, et al., 2005)**. **Eley, et al., 1993** suggested that expression of virulence factors, including haemolysins and proteases by aeromonads has been shown to be influenced by environmental temperature.

In the present case the examined bird suffered from severe Heterophys heterophys infection. Heterophyiosis is an intestinal illness caused by infection with the heterophyid digenetic flukes. Heterophyiosis has been reported as endemic in the Nile Delta of Egypt, where favorable conditions exist for parasite propagation including the availability and abundance of the intermediate hosts, increasing production of fish in unhygienic conditions due to frequent disposal of human wastes directly into rivers, shore marine water, and lakes (**Abdusslam et al. 1985; Abou-Basha et al. 2000**). Tilapia zilli is common in Egypt and is sometimes heavily infected with the metacercariae of heterophyid flukes, and are popularly eaten under cooked by residents. (**Khalil, 1933; Martin, 1959; Taraschewski, 1985**). The high parasite burden caused by to the daily meal of infected fish and this may has an immunosuppressive effect which enhances *Aeromonas salmonicida* bacteria to induce its virulence effect leading to sudden death. A numbers of factors, including age, immunocompetence, infection dose, underlying illness, and expression of sufficient virulence factors by the infecting organism, affect the ability of *Aeromonas* spp. to cause disease (**Nichols et al., 1996**). The isolated microorganism was found to be sensitive for colistin sulphate, doxycycline, cefotaxime, gentamycin, norfloxacin, enrofloxacin, nitrofurantoin and sulfamethxazole/ trimethoprim while was resistant to amoxicillin, cefradine, and erythromycin. **Sreedharan, et al. (2012)** found that all the examined aeromonas isolates displayed 100% susceptibility to trimethoprim and aminoglycosides while showed 100% resistance to oxytetracycline, tetracycline and doxycycline. Recently antibiotic resistant *A. salmonicida* strains have been recognized as a serious concern owing to their potential health risk to humans and animals **(Amos 2011).** The isolation of multi-resistant aquatic Aeromonas species warrant the need to take proper measures to prevent the introduction of resistant Aeromonas aqua culture sources used by humans, as the contact with contaminated water and fish may result in resistance gene transfer from fish to fish-eating birds and human intestinal microbiota.

Conclusion:

This is the first demonstration of Aeromonas salmonicida associated with mortality in a wild bird in Egypt. The significance of Aeromonas salmonicida infection in wild birds, and its implications for poultry and captive bird health, is currently unknown. Also this report concerns with the ability of nonpathogenic organisms to change their host preference, virulence and sensitivity resistant especially in immune-compromised host to cross all the boundaries. Also, such antibiotic resistant aeromonads, which are aquatic pathogens can multiply within fresh water ornamental fish culture systems obviously and may turn out to be a threat to wild life in addition to public health.

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